Cancer Biol Med 2017. doi: 10.20892/j.issn.2095-3941.2016.0086

REVIEW

Roles of Rap1 signaling in tumor cell migration and invasion

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ABSTRACT Ras-associated protein-1 (Rap1), a small GTPase in the Ras-related protein family, is an important regulator of basic cellular functions (e.g., formation and control of cell adhesions and junctions), cellular migration, and polarization. Through its interaction with other proteins, Rap1 plays many roles during cell invasion and metastasis in different cancers. The basic function of Rap1 is straightforward; it acts as a switch during cellular signaling transduction and regulated by its binding to either guanosine triphosphate (GTP) or guanosine diphosphate (GDP). However, its remarkably diverse function is rendered by its interplay with a large number of distinct Rap guanine nucleotide exchange factors and Rap GTPase activating proteins. This review summarizes the mechanisms by which Rap1 signaling can regulate cell invasion and metastasis, focusing on its roles in integrin and cadherin regulation, Rho GTPase control, and matrix metalloproteinase expression.

KEYWORDS Tumor; metastasis; Rap1; RapGEFs; RapGAPs

Introduction

Cell migration and tumor metastasis are responsible for up to 90% of cancer-associated mortality[1]. Ras-associated protein-1 (Rap1) plays important roles in the regulation of multiple key events in tumor cell migration, invasion, and metastasis. Rap1, a member of the 21-kilodalton Ras-like small GTPase family, can bind to either guanosine triphosphate (GDP) or guanosine diphosphate (GDP) and is modulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs)[2,3]. Rap1 shares a high degree of sequence identity (53%) with Ras protein[4] and can revert the phenotype of K-Ras-transformed cells[5]. Consistent with this observation, overexpressed Rap1V12, a constitutively active form of Rap1 (Rap1GTP), inhibits lysophosphatidic acid (LPA)-induced Ras-dependent ERK activation[6]. However, Rap1 can also activate B-Raf and ERKs in a manner independent and distinct of Ras[7]. The many roles of Rap1 include its participation in regulation of integrin- and cadherin-mediated cell adhesion in response to various membrane receptors[8] and regulation of both the recycling, avidity, and affinity of integrins by modulating an inside-out activation process[9-11]. Rap1 activation may promote the formation of cadherin-mediated cell-cell contacts through inside-out regulation[12] or cell-cell contact-induced E-cadherin-mediated outside-in signaling[13].

Regulation of Rap1 activity is primarily controlled by RapGEFs and GAPs (**Figure 1**). The dissociation rate of nucleotides from Rap1 is slow; however, GEFs accelerate this exchange reaction by several orders of magnitude[14]. Given that GEFs weaken the association between Rap1 and nucleotides, increases in GTP-bound forms over GDP-bound forms are caused by the higher intracellular concentration of GTP than GDP by approximately ten times[15]. GEFs contain a catalytic CDC25 homology domain and show selective activity for Rap1, although some GEFs can interact with other small G proteins[16]. This modulation of nucleotide binding of GEFs allows GEFs to respond to diverse stimuli, resulting in spatiotemporal regulation of Rap1. For example, RapGEFs, such as Epac1 and Epac2, are directly regulated by the secondary messenger cAMP, which controls local Epac-Rap1 signaling through its cellular distribution. Epac1 activation triggers the relocalization of Epac1 to the plasma membrane, activating membrane-localized Rap1 and enhancing integrin-mediated cell adhesion[17]. Another RapGEF, C3G, is regulated through post-translational modifications by Src and interacts with adaptor proteins of the Crk family upon activation of several receptors, including

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Received October 24, 2016; accepted December 7, 2016.
Available at www.cancerbiomed.org

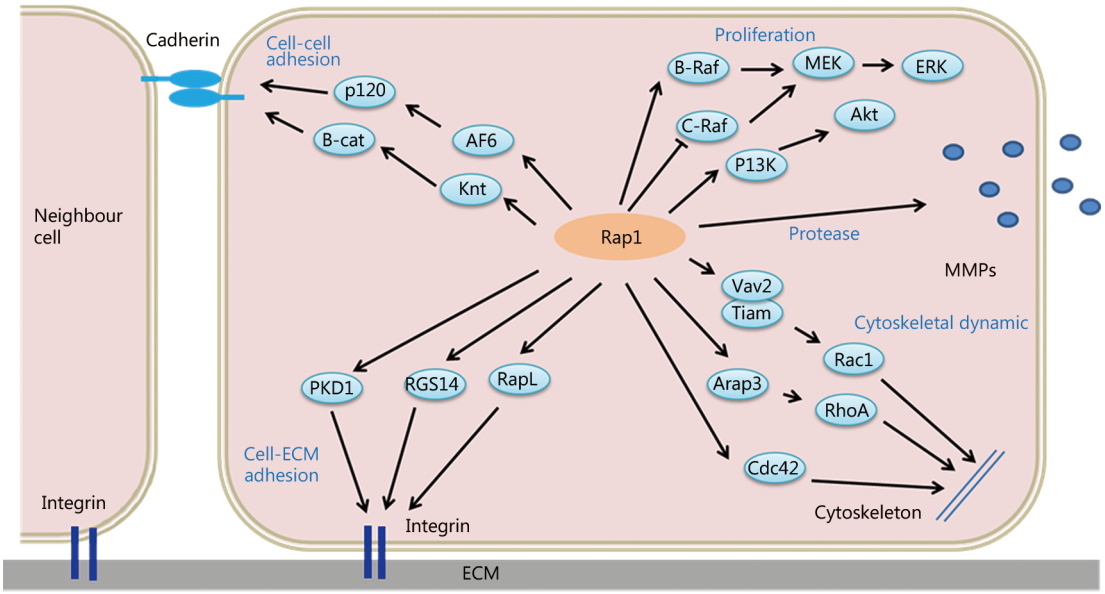

Figure 1 Mechanisms by which Rap1 signaling controls tumor cell invasion and metastasis. Rap1 signaling regulates integrin- or cadherin-mediated cell adhesion, expression levels of proteases (e.g., matrix metalloproteinase), and cytoskeletal changes, which are linked to tumor cell proliferation, invasion, and metastasis.

platelet-derived growth factor receptor and insulin receptor[18-20]. Rap1-bound GTP is efficiently hydrolyzed into GDP in the presence of RapGAPs, which accelerate the GTP hydrolysis reaction by several orders of magnitude.

Two families of Rap1-specific GAPs exist: the Rap1GAP and SIPA1 families[21]. The mechanism through which all GAPs catalyze GTP hydrolysis primarily depends on the stabilization of the catalytic machinery of G protein through insertion of a catalytic side chain into the nucleotide-binding pocket, an arginine side chain for RasGAPs and asparagine side chain for RapGAPs[22]. Through differentially distributed subcellular features, such as protein-protein interactions and epigenetic modifications, RapGAPs target different Rap1-dependent signaling complexes and consequently perform distinct cellular functions. For example, Rap1GAP is recruited from the cytosol to the plasma membrane by its interaction with $G\alpha_z$, which is activated by G protein coupled receptors[23]. E6 oncoprotein binds to SIPA1L1 (E6TP1) and targets it for degradation, resulting in deregulation of Rap1 activity[24]. In melanoma cells, Rap1GAP is downregulated via promoter methylation, promoting Rap1 activation, ERK phosphorylation, and cell proliferation and survival[25].

Moreover, the diversity of cellular functions regulated by small G proteins is determined by the distinct downstream effectors of these proteins. The effectors of Rap1 include the adaptor proteins AF-6, RAPL, Ezrin, Rasip1, Radil, Krit1, RacGEFs (e. g. , Tiam1 and Vav2), and RhoGAPs, including RA-RhoGAP and Arap3[26-31], which contribute to the regulation of Rap1-dependent cellular functions, such as cell adhesion, junction, migration, and polarization. RAPL deficiency has been speculated to significantly reduce the ability of chemokine-stimulated lymphocytes to adhere to ICAM and migrate into peripheral lymph nodes and spleen[26]. AF-6 interacts with p120 catenin and inhibits E-cadherin endocytosis in a Rap1-dependent manner[27], affecting E-cadherin-mediated cell-cell adhesion. Rasip1 mediates Rap1-induced cell spreading without affecting adhesion; it induces junctional tightening via interaction with Radil[28]. Concomitantly, Rap1 promotes translocation of Radil from cytoplasm to plasma membrane, and Radil overexpression increases cell adhesion[29]. Rap1 interacts with Tiam1 and Vav2 without affecting their catalytic activity but in turn activates Rac and CDC42, regulating cell polarization and movement[30,31]. Furthermore, the Rap1 effector B-Raf can mediate ERK activation, and regulation of PI3K/Akt by Rap1 is an important mechanism in the control of cell survival and proliferation[32] (**Figure 1**).

Tumor cell migration, invasion, and metastasis: roles of Rap1 signaling and its regulators

The diverse roles of Rap1 in the regulation of normal cell growth are translated into several distinct activities in tumor

cell development. Rap1 demonstrates distinct actions during metastasis depending on the assay employed and cancer type studied (**Table 1**) based on standard assays used to determine the roles of Rap1 include overexpression of wild-type Rap1 or its active mutants (Rap1V12 or Rap1E63), the use of extracellular stimuli, such as HGF, TGFβ, EGF, or cAMP analogs, and the use of siRNAs and the pharmacological inhibitor GGTI-298, followed by assessment of the invasive capacity of tumor cells by means of scratch and Transwell assays *in vitro* or xenograft models *in vivo*. Active Rap1 inhibits tumor invasion and metastasis in bladder, lung, and brain[33,34], whereas it has the opposite effect in melanoma, leukemia, breast cancer, esophageal squamous cell carcinoma, head and neck squamous cell carcinoma (HNSCC), pancreatic carcinoma, and non-small cell lung carcinoma[35-40]. Rap1 activation promotes the adhesion of lymphoma cells to endothelial cells and its subsequent transmigration into the hematopoietic system, through which lymphoma cells spread to distant organs[39]. Moreover, Rap1E63 contributes to the invasive ability of prostate cancer cells[41], whereas Rap1V12 suppresses prostate cancer metastasis[42]. Additionally, both Rap1V12 and Rap1GAP impair the migratory and invasive abilities of melanoma cells[39], whereas the two isoforms of Rap1, Rap1A, and Rap1B exert the opposite effect on cell motility in glioma[43,44]. These manifold phenotypes reflect the multiple signaling pathways that exist downstream of Rap1.

Similar to Rap1, which plays diverse roles in tumor metastasis, Rap1 regulators are pleiotropic (**Table 2**). Overexpression of the Rap1 activator DOCK4 suppresses invasion of mouse osteosarcoma cells[45]. Targeted shRNA-mediated EPAC1 inhibition reduces pancreatic cancer cell migration and invasion[46]. Stable expression of a non-degradable mutant of RAPGEF2 in breast cancer cells blocks tumor invasion and metastasis[47]. Rap1GAP inhibits tumor cell invasion in pancreatic carcinoma, thyroid carcinoma, melanoma, renal carcinoma, and colon cancer[48-50]; however, increased expression of Rap1GAP induces cell invasion in leukemia[51]. High expression of SIPA1 promotes tumor invasion and metastasis in prostate cancer, melanoma, and breast cancer[52,53] In colon cancer, downregulation of endogenous SIPA1 increases the invasive ability of cells[54]. This finding is inconsistent with the result for ovarian cancer, wherein C3G/Rap1 signaling promotes cell invasion, whereas Rap1GAP does not affect cell mobility[55,56]. Most of the studies included in **Table 2** also assessed the role of Rap1 and the effect of GEFs and GAPs on tumor invasion and metastasis. Exceptions are the study on Rap1GAP in pancreatic carcinoma[49] and SIPA1 in melanoma and colorectal carcinoma[52,54]; these studies did not assess whether Rap1 is involved in the observed cellular changes.

Other potential functions of Rap1 GEFs and GAPs in addition to their regulatory role on Rap1 activity cannot be ruled out. A recent study demonstrated that nuclear SIPA1 could activate integrin β1 promoter and promote breast cancer cell invasion in a Rap1-independent manner[53]. Moreover, the opposite influences of Rap1GAP and SIPA1 on regulation of melanoma cell invasion imply that there exist multiple mechanisms through which Rap1GAPs can affect cell migration and invasion. Several independent investigations have shown that the Rap1 GEF PDZ-GEF2 promotes tumor cell invasion in colon cancer, whereas Rap1GAP and SIPA1 suppresses cancer cell invasion[54,57]. This finding suggests a potential central role of Rap1 signaling and Rap1 signaling partners in colorectal carcinoma metastasis, and that the function of the Rap1 signaling proteins in tumor metastasis is very complex and mediates the effect of a host of other cellular and tissue-specific factors.

Dissemination of tumor cells from the original tumor mass involves a breakdown of cell-cell adhesion. Tumor cell migration is promoted by disruption of the extracellular matrix to form a proteolytic microtrack. Rap1 signaling participates in several processes that contribute to these events (**Figure 2**), as outlined below.

Rap1 signaling regulates cell adhesion

Rap1 signaling regulates integrins and cadherins, which play important roles in cell adhesion to ECM and in cell-cell adhesion[58]. In lung cancer, cAMP-induced Epac-Rap activation suppresses TGFβ- and HGF-stimulated cell migration by enhancing cell-cell adhesion[34]. JAM-A drives breast cancer cell migration and adhesion through activation of Rap1 and integrin β1 and formation of a complex between JAM-A, AF-6, and PDZ-GEF2[36]. Disrupting the balance in Rap1 activity in melanoma cells via expression of Rap1V12 or Rap1GAP impairs cell adhesion and migration via the FAK- and integrin-dependent pathways[39]. Given that both Rap1-specific GAPs Rap1GAP and SIPA1 inhibit cell adhesion to ECM, concluding that Rap1 plays a role in the regulation of cell adhesion is reasonable[25,52]. In prostate cancer cells, SIPA1 promotes tumor cell invasion and metastasis at least partially by inhibiting Rap1-mediated cell adhesion to ECM[42]. Reduced cell-cell adhesion is required for individual cell dissemination and invasion at the leading edge of the tumor mass during epithelial mesenchymal transition (EMT), and mesenchymal-migrating tumor cells require strong cell-to-ECM adhesion, whereas amoeboid movement does not[58]. In terms of the specific role of Rap1 in regulating integrin activation and integrin-mediated cell adhesion, Rap1 forms a complex containing talin combined with RIAM, which

Table 1 Rap1 in tumor cell invasion and metastasis

	Tumor types	Function	Signaling molecules	Cell lines	Methods used		Reference
					In vitro	*In vivo*	
Rap1	Melanoma	8CPT-2OMe-cAMP induces Rap1 activation and thus promotes cell migration.	Integrin αvβ3, ERK	A375, MeWo	Transwell	/	[35]
Rap1	Melanoma	Rap1 and RIAM are required for melanoma cell invasion.	RIAMc, Vav2-RhoA-ROCK-MLC, ERK, Akt	BLM	Transwell	/	[59]
Rap1	Lung cancer, RCC	8CPT-2OMe-cAMP treatment and Rap1 activation inhibit TGFβ- or HGF-induced cell scattering.	/	A549, RCC10	Live cell microscopy	/	[34]
Rap1	Breast cancer	Rap1 activation is required for cell migration.	Integrin β1	MCF7	Transwell	/	[36]
Rap1	HNSCC	Rap1 enhances β-catenin-dependent cell invasion	β-catenin, T-cell factor, MMP7	UM-SCC-1	Transwell	/	[75]
Rap1	Pancreatic cancer	Rap1 activation is required for EGFR-mediated invasion and metastasis *in vitro* and *in vivo*.	p130CAS/Nck1	FG, BXPC3	Transwell	Chick embryo metastasis model	[38]
Rap1	Leukemia	Rap1 activation is required for adherence to endothelial cells and transendothelial migration.	/	A20	Transwell	/	[39]
Rap1E63	Prostate adenocarcinoma	Rap1E63 increases cell migration and invasion.	Integrins α4, β3, αvβ3	PC3	Transwell	Xenograft mouse model	[41]
Rap1E63	Lung cancer	Rap1E63 promotes cell migration.	/	H1299	Scratch/ Transwell	/	[69]
Rap1V12	Bladder carcinoma	Rap1V12 inhibits cell migration.	Paxillin-Crk-DOCK180 signaling complex, Rac1	NBT-II	Random cell migration	/	[33]
Rap1V12	Melanoma	Rap1V12 or Rap1GAPII inhibits tumor cell invasion and metastasis.	FAK, integrin β1	B16F1, A375	Transwell, transendothelial migration, cell tracking	Xenograft mouse model;	[43]
Rap1V12	Prostate adenocarcinoma	Rap1V12 suppresses metastatic activity of tumor cell.	/	PC3	Transwell	Xenograft mouse model	[42]
Rap1A	ESCC	Rap1A promotes cell migration and invasion.	MMP2	EC109	Transwell	/	[76]
Rap1A	Breast cancer	Rap1A is required for cell migration and invasion induced by lysophosphatidic acid.	β-Arrestin2, IQGAP1	MDA-MB-231	Scratch/ Transwell	/	[77]
Rap1A	Glioma	Rap1A is required for PDGF-BB-stimulated chemotactic motility and 3D spheroid invasion.	/	U87	Transwell, three-dimensional spheroid invasion assay	/	[45]
Rap1B	Glioma	Rap1B suppresses migration of glioma cells.	Rac1	GNS-3314	Transwell	/	[44]
Rap1B	Leukemia	Rap1B is required for transendothelial migration of cells.	Integrin β2	CCRF-CEM	Transendothelial migration	/	[40]

RCC, renal cell carcinoma; HNSCC, head and neck squamous cell carcinoma; ESCC, esophageal squamous cell carcinoma.

Table 2 Role of Rap1 GEFs and GAPs in tumor cell invasion and metastasis

Item	Tumor types	Function	Signaling molecules	Cell lines	Methods used		References
					In vitro	*In vivo*	
DOCK4	Osteosarcoma	DOCK4 inhibits tumor cell invasion and metastasis.	Rap1	3081	/	Xenograft mouse model	46
PDZ-GEF1	Breast cancer	PDZ-GEF1 blocks cell invasion.	/	MDA-MB-231	Transwell	Zebrafish model	49
PDZ-GEF2	Colon cancer	PDZ-GEF2 enhances cell migration.	JAM-A/(AF6/PDZ-GEF2), Rap1A, Integrinβ1	SKCO-15	Scratch assay	/	58
C3G	Ovarian cancer	C3G/Rap1 pathway promotes cell invasion and metastasis.	MMP2, MMP9	SKOV3, HEY	Transwell	/	65
EPAC1	Pancreatic cancer	EPAC1 inhibition inhibits cell migration and invasion.	Rap1, Akt	AsPC-1, PANC-1	Transwell	/	47
Rap1GAP	Colon cancer	Rap1GAP impairs the ability of cells to spread and migrate on collagen IV.	Rap2	HCT116	Scratch assay	/	78
Rap1GAP	Colon cancer	Downregulation of Rap1GAP endows tumor cells with more aggressive properties.	Rap1/2, Rac1	HT-29, LoVo	Scratch assay, transwell, phagocytic tracking assays	/	50
Rap1GAP	Pancreatic cancer	Rap1GAP inhibits cell invasion and metastasis.	FAK	MiaPaCa-2	Transwell, colloidal gold random motility assays	Xenograft mouse model	61
Rap1GAP	Thyroid cancer	Rap1GAP inhibits cell migration and invasion.	Rap1, Rac	WRT, FTC133, WRO	Transwell; scratch assay	/	48
Rap1GAP	Thyroid cancer	Rap1GAP inhibits cell migration and invasion.	Rap1	TPC1; Hth83	Transwell; scratch assay	/	79
Rap1GAP	Thyroid cancer	Rap1GAP inhibits cell motility.	Rap1/2, FAK, Paxillin, Src	BCPAP, KTC-1, TPC-1	Scratch assay	/	51
Rap1GAP	Oropharyngeal SCC	Rap1GAP induces oropharyngeal SCC cell invasion and metastasis.	Rap1, MMP2, MMP9	UM-SCC-11A	Transwell	Xenograft mouse model	80
Rap1GAP	Melanoma	Rap1GAP inhibits melanoma cell migration.	Src, Rap1, ERK	LH	Transwell	/	25
Rap1GAP	Ovarian cancer	Rap1GAP does not affect migration properties of OVCA cell lines.	/	Established cell lines derived from patients	Transwell	/	65
Rap1GAP	RCC	Rap1GAP inhibits cell invasion.	Rap1	RCC7	Transwell	/	57
Rap1GAP	RCC	Rap1GAP inhibits cell invasion.	Integrins, cadherins	Caki-1, SN12C	Transwell	/	81
Rap1GAP	Leukemia	Rap1GAP promotes leukemia cell invasion.	MMP9	HL-60	Transwell	/	52
SIPA1	Prostate cancer	SIPA1 promotes prostate cancer cell invasion and metastasis.	Brd4/ECM-related genes, Rap1	LNCaP, PC3	Transwell	Xenograft mouse model	42
SIPA1	Melanoma	SIPA1 is required for melanoma cell migration.	/	VM-1	Transwell	/	53
SIPA1	Colon cancer	SIPA1 inhibits cell migration and invasion.	/	HT115, Caco2	Scratch assay, Transwell	/	82
SIPA1	Breast cancer	SIPA1 promotes cell migration and invasion.	Integrin β1/FAK/Akt/MMP 9	MDA-MB-231	Scratch assay, Transwell	Zebrafish model	54

SCC, squamous cell carcinoma; RCC, renal cell carcinoma.

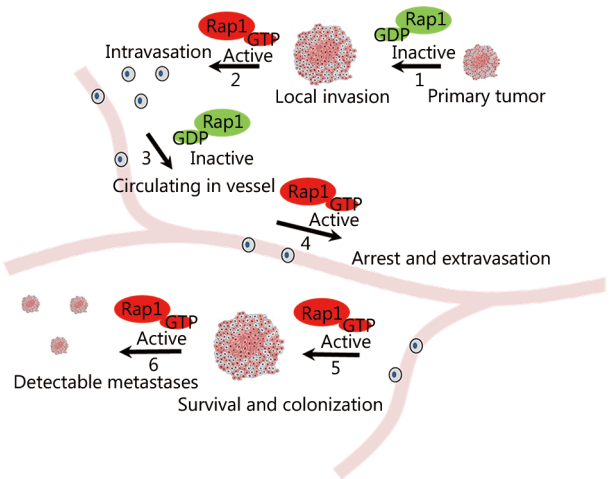

Figure 2 Dynamic change in Rap1 signaling during tumor cell invasion and metastasis. Dynamic change or cycling of Rap1 activity is required for invasive and metastatic behavior of tumor cells. For instance, while inactivation of Rap1-cadherin or integrin signaling is associated with reduced cell–cell adhesion or cell adhesion to extracellular matrix in one stage (steps 1 and 3), a separate step might entail increased Rap1 activity and cell adhesion (steps 2, 4, 5, and 6).

targets talin to integrin[59]. However, a complete description of the roles of Rap1 in mediating cell adhesion in tumor cell invasion and metastasis requires further clarification.

Rap1 signaling modulates expression of matrix metalloproteinases (MMPs)

During tumor invasion and metastasis, MMPs degrade ECM barriers, cleave and activate target proteins, and regulate cell adhesion. In HNSCCs, Rap1 promotes nuclear localization of β-catenin, which induces TCF-dependent MMP7 transcription, thereby contributing to tumor cell invasion[37]. Knockdown of C3G in ovarian cancer cells reduces MMP2 and MMP9 production and Rap1-GTP level[56]. However, in HNSCCs, overexpression of Rap1GAP increases the expression levels of MMP2 and MMP9 and the invasive capacity of cells, although the role of Rap1 in this process is unclear[62]. Overexpression of SIPA1 in prostate cancer cells reduces MMP12 expression[42]. By contrast, SIPA1 knockdown in breast cancer cells reduces MMP9 expression through the FAK/Akt pathway[53].

Rap1 signaling controls Rho GTPase-mediated regulation of cytoskeletal dynamics

Several Rho family members function in actin cytoskeleton rearrangement and consequently in modulation of cell motility. Rap1 signaling can participate in motility regulation involving Rho family proteins, particularly Cdc42, Rac1, and RhoA. Rap1 associates with RacGEFs, such as Vav2 and Tiam1, to induce translocation of Vav2 and activates Rac1 to promote cell spreading[30]. Cdc42 activation by Rap1 increases the activity of cell polarization-related protein complex, which in turn activates Rac1 through Tiam1 and subsequently enhances cell polarization[31]. Moreover, Rap1 can interact with and activates Arap3, a RhoA GAP. During tumor metastasis, Rap1 increases the ability of melanoma cell to migrate via Vav2-dependent activation of the RhoA/ROCK/MLC pathway[60]. *In vitro* overexpressed Rap1GAP inhibits Rap1, Rac1 activation, and thyroid tumor cell migration[61]. Additionally, Rap1's inhibitory effects on bladder cancer and glioma cell migration are intensified by reduced Rac1 activity[33,43]. Rap1 signaling can regulate Rho-family protein activities either positively or negatively, causing a wide range of effects on tumor cell invasion and metastasis.

Rap1 signaling controls cell proliferation

Tumor cell growth can increase tumor volume and mass, contributing to invasion via physical pushing[63]. An inhibitory effect of Rap1 signaling-related molecules on cell proliferation and invasion has been repeatedly observed; for instance, DOCK4 inhibits osteosarcoma and Rap1GAP inhibits pancreatic cancer, thyroid carcinoma, and melanoma cells[25,45,48,49]. Additionally, SIPA1 drives both cell proliferation and invasion in melanoma cells[52]. SIPA1-induced expression exerts little effect on primary tumor mass in prostate cancer but significantly increases both tumor cell invasion and metastasis, suggesting that SIPA1 promotes metastasis through mechanisms other than proliferation[42]. SIPA1 knockdown impairs the invasive capacity of breast cancer cells while it enhances their proliferation[53]. Similarly, overexpression of Rap1V12 in melanoma cells increases tumor mass but inhibits tumor metastasis *in vivo*[39]. Moreover, Rap1GAP overexpression inhibits cell growth but induces MMP2- and MMP9-mediated oropharyngeal squamous carcinomas cell invasion[51].

Regulation of Rap1 is dependent on tissue and subcellular-specific factors

Rap1 signaling can affect metastasis in different manners depending on tumor types (**Table 3**). Tissue-specific protein expression in different tumor types likely contributes to the

Table 3 Bidirectional effects of Rap1 signaling in different tumor types

Item	Metastasis-promoting tumor	Metastasis-suppressing tumor
Rap1	Breast, esophageal, glioma, HNSCC, leukemia, lung, melanoma, pancreas, prostate, ovarian	Bladder, glioma, lung, prostate
GEFs	Colon, pancreas, ovarian	Breast, osteosarcoma
GAPs	Breast, leukemia, melanoma, prostate, oropharyngeal SCC	Colon, kidney, melanoma, pancreas, thyroid

HNSCC: head and neck squamous cell carcinoma; SCC: squamous cell carcinoma.

regulation of Rap1 signaling, similar to the spatiotemporally regulated patterns of gene expression during tumor development[64]. Indeed, Rap1 has been implicated in the activation and inhibition of ERK pathway in different cell types[21]; cAMP-induced activation of Rap1 inhibits C-Raf-induced ERK activation[65]. However, in neuronal cells expressing B-Raf, activated Rap1 can directly bind to B-Raf and induces downstream ERK activation[7,66]. Additionally, over-activation or inactivation of Rap1 inhibits melanoma cell motility, suggesting that change in Rap1 activity is critical for the metastatic dissemination of melanoma cells[39]. The interaction of Rap1 signaling with tissue-specific factors may explain this considerably diverse functions of Rap1. For example, while basal level of Rap1-GTP maintains cell adhesion, insulin-like growth factor type I receptor transiently regulates Rap1 activity through C3G and Rap1GAP to promote cell movement[67].

Protein subcellular localization of Rap1 is vital to the specificity and diversity of its function[68]. Relatedly, tumor cell dissemination and invasion depends on the stability and activity of Rap1 (**Figure 2**). Rap1 phosphorylation prevents the membrane association of Rap1, resulting in cytosolic and nuclear accumulation and in subsequent decrease in Rap1-dependent cell adhesion[69,70]. In addition, Rap1 stabilizes β-catenin in the nucleus and enhances β-catenin-dependent transcription and invasion in HNSCC[37,51]. SIPA1, recruited by AF6 and co-localized with Rap1 at cell adhesion sites, inhibits endogenous Rap1GTP and integrin β1-mediated cell adhesion to fibronectin[71]. However, nuclear-localized SIPA1 activates the integrin β1 gene promoter and promotes cell invasion and adhesion (**Figure 3**)[53].

Novel targets for the prevention of metastasis: insights from related studies on Rap1 signaling

Prevention or early detection of the initial dissemination of tumor cells and secondary spread of tumor is an important goal in research aiming to find better clinical therapies[72]. In a melanoma metastasis model, six distinct Rap1-regulating molecules were used to predict the aggressive capability of melanoma cells[52]. Several inhibitors of cell motility, such as metalloproteinase inhibitor[73] and the fascin inhibitor Migrastatin[74], have been suggested to demonstrate clinical utility in preventing tumor cell dissemination and subsequent invasion and metastasis. However, formation of metastases often occurs prior to the diagnosis of cancer. The Rap1 signaling pathway offers many targets for novel clinical tools given that Rap1 affects not only cell polarity and cell adhesion but also cell proliferation and invasion. Treatment

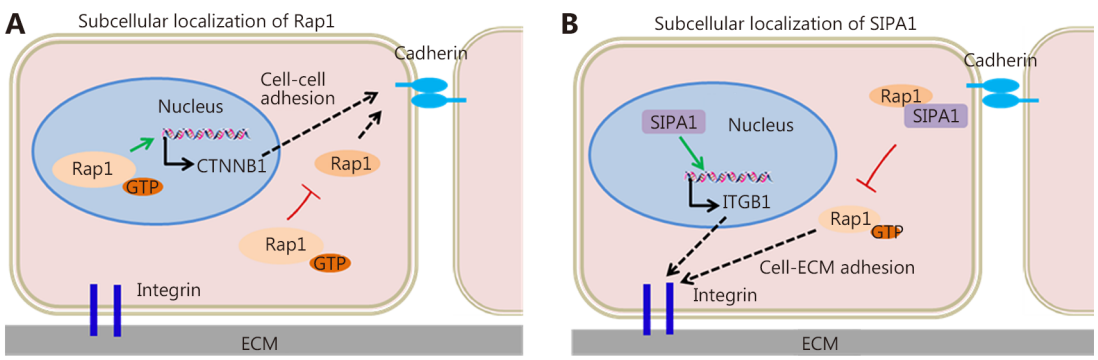

Figure 3 Subcellular localization of Rap1 and SIPA1 during tumor cell invasion and metastasis. Subcellular localization of Rap1 (A) and SIPA1 (B) contributes to their distinct functions within a cell.

with the demethylating agent 5-aza-2'-deoxycytidine induces Rap1GAP expression and reduces melanoma cell proliferation and survival[25]. In addition, treatment with 5-aza-deoxycytidine and/or the histone deacetylation inhibitor trichostatin A induces Rap1GAP expression in thyroid tumor cells, reducing cell invasion and proliferation[48,75]. Additional studies on these and other novel reagents targeting Rap1 signaling molecules are called for.

Conclusions

Rap1 signaling plays several important roles in tumor cell invasion and metastasis. The full scope of its functions remains unknown; Rap1 can induce very distinct effects depending on the tissue in which Rap1 is expressed. Therefore, the specific functions and effects of Rap1 signaling on metastasis in different tumor types remains a subject of continuing research. Additionally, many proteins contribute to the diversity in the control of tumor invasion and metastasis by Rap1 signaling, and the full panoply of factors that work with Rap1 resulting in diverse control mechanisms is not yet fully elucidated. Future works employing high throughput screening strategies to identify new molecules contributing to Rap1 signaling and real-time monitoring of Rap1 signaling during tumor invasion and metastasis are needed to further define the roles of Rap1.

Acknowledgements

This study is supported by grants from the National Natural Science Foundation of China (Grant No. 31271504 and 31471310) and the Shenzhen Science and Technology Innovation Committee, China (Grant No. JCYJ2013040 1144744187).

Conflict of interest statement

No potential conflicts of interest are disclosed.

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Cite this article as: Zhang Y, Wang R, Cheng K, Ring BZ, Su L. Roles of Rap1 signaling in tumor cell migration and invasion. Cancer Biol Med. 2017; 14: 90-9. doi: 10.20892/j.issn.2095-3941.2016.0086